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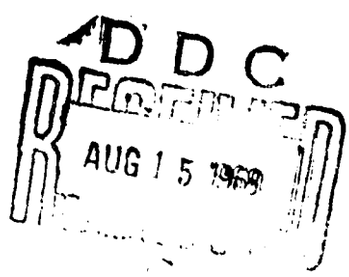

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IMMUNOLOGICAL ACTIVITY OF STAPHYLOCOCCUS FOR ANTI-ANTHRAX PRECIPITIN SERUM

II. ON THE COMMON ANTIGEN BETWEEN B. ANTHRACIS AND STAPHYLOCOCCUS

[Following is a translation of an article by Shizuo Takagi and Takeshi Baba, Department of Animal Microbiology, College of Agriculture, Osaka Prefecture University, Japanese Journal of Bacteriology, Vol. 17, 1962, pages 330-333.]

(Rec'd for publication; Feb. 3, 1962)

We have reported [1] on the common antigenicity between B. anthracis and B. staphylococcus in the previous paper observing the reaction between the anti-anthrax precipitinogen serum and the extract of heat-dried B. staphylococcus, esp, staphylococcus aureus. This paper reports the further study on the common antigen using the acid precipitation method, copper salt precipitation method, and Sevag's trichloroacetic acid method.

Maillard,[2] and Rothbard [3] have reported on the existence of common antigen between B. staphylococcus and B. tuberculosis by the hemolytic reaction of the tuberculin sensitized erythrocyts.

Experimental Methods and Results.

B. anthracis vacl. and staphylococcus aureus 209p were used. B. anthracis was cultured in agar for 18 hours and 209p for 24 hours. Each was then suspended in physiologic saline, vibration washing was repeated for 3 times, and heat-dried sample was then fractionated into four fractions as shown in Fig. 1.

(A represents anthracis fraction and B 209p)

[Explanation of Fig. 1.] Heat-dried bacteria was suspended 400 mg/ml in physiologic saline. Extraction was repeated at 37°C. N-HCl was added to the extract to adjust pH at 3.8 and kept standing still overnight at 4°C. Precipitate was washed with pH 3.8 citrate buffer and precipitate (A, 1, S, 1,) was obtained. To the supernatant, $CuSO_4$ was added to make its concentration 10% and kept standing still overnight at 4°C. Then the precipitate was washed with 10% $CuSO_4$ solution and precipitate (A-2, S-2) was obtained and the supernatant was treated with Sevag's procedure.

Five volumes of methanol was added to one volume of Gel part treated with Sevag's procedure to dissolve the precipitate, then trichloroacetic acid was added to make 4% solution and precipitate (A-3, S-3) was

obtained. On the other hand the supernatant after Sevag's procedure (A-4, S-4) was also obtained. The final titer of precipitation reaction and general chemical specialties of B. anthracis' (A-1, -2, -3, -4) and aureus 209p's (S-1, -2, -3, -4) four fractions to anti-anthrax serum and anti-209p serum are shown in Table 1. The fractions of S-1, -2, -3 of 209p reacted to anti-anthrax serum as shown in Table 1. The fraction of A-1, and -3 of anthrax reacted to anti-209p serum. Therefore, the existence of a common antigen in the fractions of A-1, -3, S-1, and S-3.

For the second step, an absorption test and agar dispersion method were applied to observe the antigen relation between anthrax and 209p fractions. The results are shown in Table 2. and Fig. 2. There was a common antigen among each fraction of B. anthracis and another common antigen among A-1, -3, -4, and A-3, -4. A specific polysaccharide antigen existed in A-4.

There were common antigens among the fractions of 209p. Among S-1, -3, -4, and S-1, -4, there was another common antigen. In S-4, there was a specific antigen.

A common antigen was found among A-1, -2, -3, S-1, S-2, and S-3, however, this reaction was not found in the absorption test. This is probably due to the decrease of antigen titer by some unknown reason other than antigen antibody reaction.

Table 1. Properties of the fractions of B. anthracis and 209p strain.

properties / fractions		precipitin titers					
		anti-anthrax serum		Anti-209p serum		normal rabbit serum	
B. anthracis:							
	A-1	++	8,000	++	4,000	-	0
	-2	±	1,000	±	1,000	-	0
	-3	++++	32,000	+++	8,000	-	0
	-4	++++	512,000	-	0	-	0
209p:	S-1	+++	4,000	+++	8,000	-	0
	-2	+	4,000	+++	8,000	-	0
	-3	+++	8,000	+++	8,000	-	0
	-4	-	0	++++	64,000	-	0

chemical properties					properties / fractions	
Molisch	Biuret	Ninhydrin	D.P.N.	Orcin-HCl		
-	++	++	+	±	A-1	B. anthracis
-	±	+	+	-	-2	
-	++	++	-	+	-3	
++	-	+	-	-	-4	
-	++	++	-	++	S-1	209p
-	∓	+	∓	++	-2	
-	+	+	-	++	-3	
++	-	+	-	±	-4	

Note: 1) 1/10% solution was used for precipitation and qualitative reaction.
2) ++++, +++, ++, +, -, [±, ∓, also] mean the strength of reactions.
3) Normal rabbit serum means preimmunized serum.

Table 2. Antigen relation of the fractions of B. anthracis and 209p.

anti-sera	absorbers	reactors			
		A-1	A-2	A-3	A-4
anti-anthrax serum	A-1	-	-	+++	+
	-2	++	-	+++	++
	-3	-	-	-	+
	-4	-	-	-	-
anti-sera	absorbers	S-1	S-2	S-3	S-4
anti-209p serum	S-1	-	-	-	++++
	-2	-	-	++	++++
	-3	-	-	-	+++
	-4	-	-	-	-

Table 3. Antigen relation between fractions of A-1, -3, S-1, and S-3.

anti-sera	absorbers	reactors			
		A-1	A-3	S-1	S-3
anti-anthrax serum	A-1	-	+++	-	-
	-2	-	-	-	-
	-3	++	++++	-	-
	-4	++	++++	-	-

anti-	S-1	-	+^{d}	-	-
209p	-2	-	-	++^{d}	-
serum	-3	-	+	++++	+++^{d}
	-4	-	-	++++	++

Since the existence of a common antigen between B. anthracis and 209p strain among A-1, -3, S-1, and S-3 was expected, the antigen relation of these 4 fractions was examined further by absorption test and agar dispersion method.

As shown in Table 3, and Fig. 4. each fraction reacted to anti-anthrax serum and to 209p serum. Moreover, intra-agar common reaction belts were observed as shown in the diagram. However, as shown in the table, such a reactor as observed in A-3, which is different from those in the other three fractions was expected to be contained in S-1 and S-3, although the reaction to S-1, and S-3 was negative. The negative results may due to its small quantity.

The existence of B.P.B. 2 to 4 positive protein in A-1, -3, S-1, and S-3 was found by paper electrophoresis (Veronal buffer pH 8.6, i=0.1, 300 V, 1 mA/cm, 8 hrs).

Discussion and Summary

The existence of various common antigens in the living bacteria bodies was thought probable according to the study pursued; the common antigen between B. anthrecis and 209p strain, although thermolabile proteins were not studied.

The common antigen between A-1 and S-1, and A-3 and S-3 also appeared common to the 4 fractions by absorption test and agar dispersion method. However, it is difficult to discuss in detail without the separation of each antigen as a single substance. It seems likely that there was a partial contamination of Sevag's trichloroacetic acid fraction into HCl fraction and vice versa, since both fractionations were done near pH 3.8. Therefore, a substance reacted positively to the absorption test and the agar dispersion method could be the same one existing in all of the 4 fractions.

Recently, Cohen[4] and co-worker has reported the existence of a substance reacted to the HCl-ether-CCl_3COOH precipitate of staphylococcus aureus in the non-immunized normal rabbit serum. This is a phenomena only found in a kind of rabbit and it is naturally supposed that the existence of such substance as an acquired product of natural infection, or as an inborn portion of prophylactic system.

The antiserum we used was pre-examined and cleared that it did not have any reactivity to the extract of heat-dried staphylococcus aureus.

Summarizing the above mentioned results:-

(1). Four fractions were obtained by HCl, $CuSO_4$, and Sevag's CCl_3COOH precipitation method. A common antigenic substance was observed existing in the HCl, and CCl_3COOH precipitated fraction by absorption test and agar dispersion method.

(2). The common antigenic substance was thought to be a protein substance having Molisch negative, Biuret positive and Ninhydrin positive reactions. No common antigenic substance was observed in the fractions A-4, and S-4, which were supposed to be polysaccharide.

(3). The fractions A-1, -3, S-1, and S-3, containing common antigenic substances, showed 2 to 4 kinds of antigens (reactor). The common antigenic substance is thought to have 1 to 4 substances among them.

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2). Maillard, E. R.: Am. Rev. Tuberc., 66, 762, 1952.

3). Rothbard, S.: Ann. Rev. Med., 5, 339, 1954.

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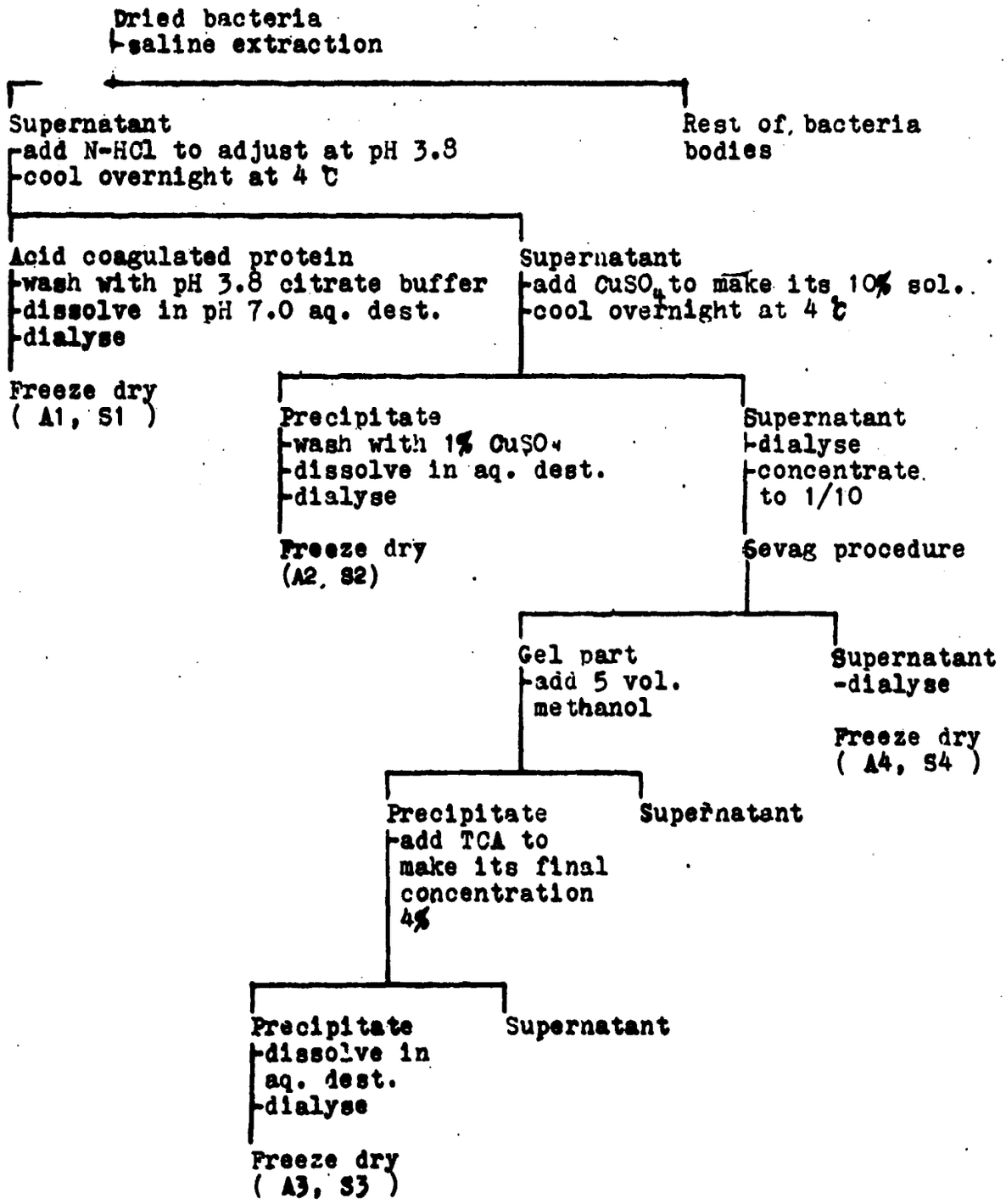
Fig. 1. Method of precipitation of B. anthracis and 209p strain.



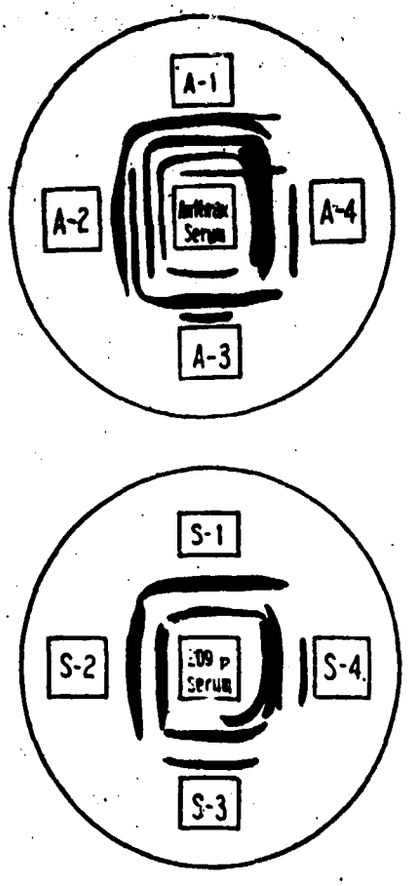


Fig 2. Intra-agar precipitation reaction of the fractions of B. anthracis and 209p strain.

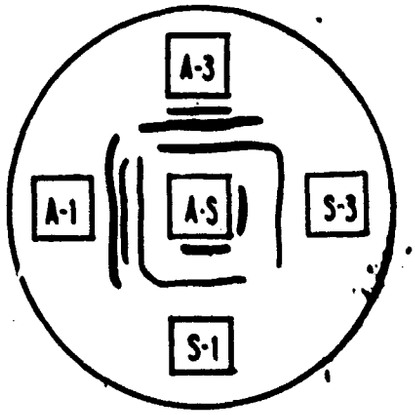


Fig 3. Intra-agar precipitation reaction of fractions of A-1, -3, S-1, and S-3.